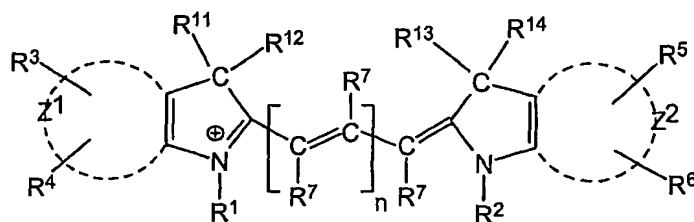


Claims

1. A compound of formula (I):



(I)

wherein:

groups  $R^3$  and  $R^4$  are attached to the  $Z^1$  ring structure and groups  $R^5$  and  $R^6$  are attached to the  $Z^2$  ring structure, and  $n = 1, 2$  or  $3$ ;

$Z^1$  and  $Z^2$  independently represent the carbon atoms necessary to complete a one ring, or two-fused ring aromatic system;

at least one of groups  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  and  $R^7$  is the group  $-E-F$  where  $E$  is a single bond or a spacer group having a chain from 1–20 linked atoms selected from the group consisting of carbon, nitrogen and oxygen atoms and  $F$  is a target bonding group;

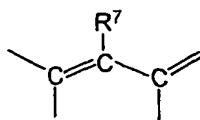
one or more of groups  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  are independently selected from the group  $-(CH_2)_k-W$ , where  $W$  is sulphonic acid or phosphonic acid and  $k$  is an integer from 1 to 10;

when any of groups  $R^1$  and  $R^2$  is not said group  $-E-F$ , said remaining groups  $R^1$  and  $R^2$  are independently selected from  $C_1 - C_6$  alkyl, benzyl either

unsubstituted or substituted with sulphonic acid, and the group  $-(CH_2)_k-W$ , where  $W$  and  $k$  are hereinbefore defined;

when any of groups  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  is not said group  $-E-F$ , said remaining groups  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  are independently selected from hydrogen and sulphonic acid;

when any of groups  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  is not said group  $-(CH_2)_k-W$ , said remaining groups  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  are independently  $C_1 - C_6$  alkyl; remaining groups  $R^7$  are hydrogen or two of  $R^7$  together with the group,



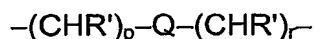
- 5 form a hydrocarbon ring system having 5 or 6 atoms.
2. A compound according to claim 1 wherein at least two of  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  are independently  $-(CH_2)_k-W$  wherein  $W$  and  $k$  are hereinbefore defined.
- 10 3. A compound according to claim 1 wherein one of groups  $R^{11}$  and  $R^{12}$  and one of groups  $R^{13}$  and  $R^{14}$  is the group  $-(CH_2)_k-W$  wherein  $W$  and  $k$  are hereinbefore defined; and remaining groups  $R^{11}$  or  $R^{12}$  and  $R^{13}$  or  $R^{14}$  are  $C_1-C_6$  alkyl.
- 15 4. A compound according to any of claims 1 to 3 wherein  $W$  is sulphonic acid.
5. A compound according to any of claims 1 to 3 wherein  $-(CH_2)_k-W$  is
- 20 selected from  $-(CH_2)_3-SO_3H$  and  $-(CH_2)_4-SO_3H$ .
6. A compound according to any of claims 1 to 5 wherein  $Z^1$  and  $Z^2$  are selected from phenyl and naphthyl moieties.
- 25 7. A compound according to any of claims 1 to 6 wherein said target bonding group  $F$  comprises a reactive group for reaction with a functional group on a target material, or a functional group for reaction with a reactive group on a target material.
- 30 8. A compound according to claim 7 wherein said reactive group is selected from carboxyl, succinimidyl ester, sulpho-succinimidyl ester, isothiocyanate, maleimide, haloacetamide, acid halide, hydrazide, vinylsulphone, dichlorotriazine and phosphoramidite.

9. A compound according to claim 7 wherein said functional group is selected from hydroxy, amino, sulphhydryl, imidazole, carbonyl including aldehyde and ketone and thiophosphate.

5 10. A compound according to any of claims 1 to 6 wherein said target bonding group F comprises an affinity tag.

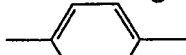
11. A compound according to any of claims 1 to 10 wherein said spacer group E is selected from:

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where Q is selected from:  $-\text{CHR}'-$ ,  $-\text{NR}'-$ ,  $-\text{O}-$ ,  $-\text{CR}'=\text{CR}'-$ ,  $-\text{C}(\text{O})-\text{NR}'-$  and  $-\text{C}(\text{O})-\text{O}-$ ; R' is hydrogen or  $\text{C}_1 - \text{C}_4$  alkyl, p is 0 – 5 and r is 1 – 5.

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12. A compound according to claim 11 wherein Q is selected from:  $-\text{CHR}'-$ ,  $-\text{C}(\text{O})-\text{NH}-$  and ; where R' is hereinbefore defined.

13. A compound according to any of claims 1 to 6 wherein said group  $-\text{E}-\text{F}$   
20 comprises a carboxypentyl group.

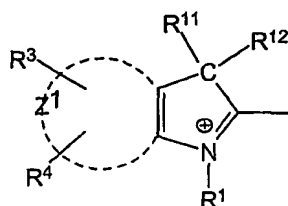
14. A compound according any of claims 1 to 13 selected from:

- i) 2-((1*E*,3*E*,5*E*)-5-[1-(5-carboxypentyl)-3-methyl-5-sulpho-3-(4-sulphobutyl)-1,3-dihydro-2*H*-indol-2-ylidene]penta-1,3-dienyl)-1-ethyl-3-methyl-3-(4-sulphobutyl)-3*H*-indolium-5-sulphonate;
- 25 ii) 2-((1*E*,3*E*,5*E*)-5-[1-(5-carboxypentyl)-3-methyl-5-sulpho-3-(4-sulphobutyl)-1,3-dihydro-2*H*-indol-2-ylidene]penta-1,3-dienyl)-3-methyl-1,3-bis(4-sulphobutyl)-3*H*-indolium-5-sulphonate;
- 30 iii) 2-((1*E*,3*E*,5*E*,7*E*)-7-[1-(5-carboxypentyl)-3-methyl-5-sulpho-3-(4-sulphobutyl)-1,3-dihydro-2*H*-indol-2-ylidene]hepta-1,3,5-trienyl)-1-ethyl-3-methyl-3-(4-sulphobutyl)-3*H*-indolium-5-sulphonate;

- iv) 2-((1*E*,3*E*,5*E*,7*E*)-7-[5-(carboxymethyl)-3-methyl-1,3-bis(4-sulphobutyl)-1,3-dihydro-2*H*-indol-2-ylidene]hepta-1,3,5-trienyl)-1-ethyl-3-methyl-3-(4-sulphobutyl)-3*H*-indolium-5-sulphonate; and
- v) 1-benzyl-2-((1*E*,3*E*,5*E*)-5-[1-(5-carboxypentyl)-3-methyl-5-sulpho-3-(4-sulphobutyl)-1,3-dihydro-2*H*-indol-2-ylidene]penta-1,3-dienyl)-3-methyl-3-(4-sulphobutyl)-3*H*-indolium-5-sulphonate.

15. A method for preparing a compound according to any one of claims 1 to 14, the method comprising:

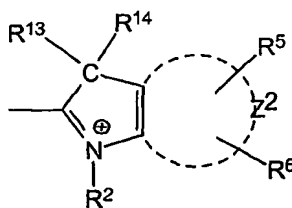
- a) reacting a first intermediate compound having the formula (A):



(A)

wherein  $Z^1$ ,  $R^1$ ,  $R^3$ ,  $R^4$ ,  $R^{11}$  and  $R^{12}$  are hereinbefore defined;

- b) a second intermediate compound which may be the same or different from the first intermediate compound and having the formula (B):



(B)

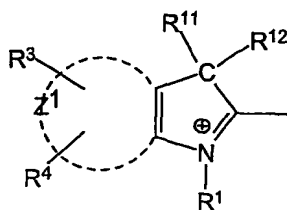
wherein  $Z^2$ ,  $R^2$ ,  $R^5$ ,  $R^6$ ,  $R^{13}$  and  $R^{14}$  are hereinbefore defined; and

- c) a third compound (C) suitable for forming a linkage between the first and second compounds;

provided that at least one of the groups  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  is the group -E-F, where E and F are hereinbefore defined; and provided that one or more of groups  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  are independently selected from the group

$-(CH_2)_k-W$ , where W is selected from sulphonic acid and phosphonic acid groups and k is an integer from 1 to 10.

16. A compound of formula:



wherein:

groups  $R^3$  and  $R^4$  are attached to the  $Z^1$  ring structure, wherein  $Z^1$  is hereinbefore defined;

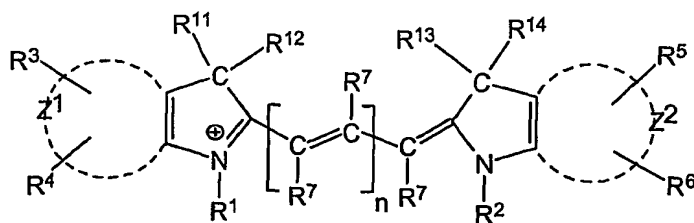
at least one of the groups  $R^1$ ,  $R^3$  and  $R^4$  is the group  $-E-F$  where E and F are hereinbefore defined;

at least one of groups  $R^{11}$  and  $R^{12}$  is the group  $-(CH_2)_k-W$ , where W is selected from sulphonic acid and phosphonic acid groups and k is an integer from 1 to 10.

17. A compound according to claim 16 wherein  $-(CH_2)_k-W$  is selected from  $-(CH_2)_3-SO_3H$  and  $-(CH_2)_4-SO_3H$ .

18. A method for labelling a target component, the method comprising:

i) contacting said component with a compound of formula (I):



(I)

wherein:

groups  $R^3$  and  $R^4$  are attached to the  $Z^1$  ring structure and groups  $R^5$  and  $R^6$  are attached to the  $Z^2$  ring structure, and  $n = 1, 2$  or  $3$ ;

$Z^1$  and  $Z^2$  independently represent the carbon atoms necessary to complete a one ring, or two-fused ring aromatic system;

- 5 at least one of groups  $R^1, R^2, R^3, R^4, R^5, R^6$  and  $R^7$  is the group  $-E-F$  where  $E$  is a single bond or a spacer group having a chain from 1–20 linked atoms selected from the group consisting of carbon, nitrogen and oxygen atoms and  $F$  is a target bonding group;

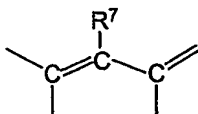
- 10 one or more of groups  $R^{11}, R^{12}, R^{13}$  and  $R^{14}$  are independently selected from the group  $-(CH_2)_k-W$ , where  $W$  is sulphonic acid or phosphonic acid and  $k$  is an integer from 1 to 10;

when any of groups  $R^1$  and  $R^2$  is not said group  $-E-F$ , said remaining groups  $R^1$  and  $R^2$  are independently selected from  $C_1 - C_6$  alkyl, benzyl either unsubstituted or substituted with sulphonic acid, and the group  $-(CH_2)_k-W$ ,

- 15 where  $W$  and  $k$  are hereinbefore defined;

when any of groups  $R^3, R^4, R^5$  and  $R^6$  is not said group  $-E-F$ , said remaining groups  $R^3, R^4, R^5$  and  $R^6$  are independently selected from hydrogen and sulphonic acid;

- 20 when any of groups  $R^{11}, R^{12}, R^{13}$  and  $R^{14}$  is not said group  $-(CH_2)_k-W$ , said remaining groups  $R^{11}, R^{12}, R^{13}$  and  $R^{14}$  are independently  $C_1 - C_6$  alkyl; remaining groups  $R^7$  are hydrogen or two of  $R^7$  together with the group,



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form a hydrocarbon ring system having 5 or 6 atoms; and

- ii) incubating said fluorescent dye with said component under conditions suitable for binding to and thereby labelling said component.

- 30 19. A method according to claim 18 wherein said component is selected from the group consisting of antibody, lipid, protein, peptide, carbohydrate, nucleotides which contain or are derivatized to contain one or more of an amino, sulphydryl, carbonyl, hydroxyl and carboxyl and thiophosphate groups,

and oxy or deoxy polynucleic acids which contain or are derivatized to contain one or more of an amino, sulphhydryl, carbonyl, hydroxyl, carboxyl and thiophosphate groups, microbial materials, drugs, hormones, cells, cell membranes and toxins.

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